REMARKS

Entry of the foregoing, reexamination and reconsideration of the subject application, as amended, pursuant to and consistent with 37 C.F.R. §1.112, are respectfully requested in light of the remarks which follow

Status of the Claims

Claims 29-35 and 40-44 are pending, with claim 29 being independent. Claims 29, 35, 40 and 41 are amended herein. Support for the claim amendments and new claim can be found throughout the specification and claims as filed, such as on page 6, first paragraph. As such, no new matter has been added.

Applicants respectfully request the Examiner to reconsider and withdraw the outstanding rejections in view of the foregoing amendments and the following remarks.

Priority

The Office requests that the application data sheet be amended to recite the claim for priority. A revised application data sheet is submitted herewith.

Objections

Claim 35 is objected to for reciting "CD-3". Claim 35 is amended to recite "CD3" as disclosed in the specification.

The specification is objected to for purportedly failing to provide basis for subject matter of claims 29, 32, and 33. With regard to cells obtained from biopsies, Applicants refer, for example, to the in vitro and in vivo studies of CAPRI cells, set forth at paragraphs [0054] through [0058].

With regard to the recitation of "about 30 million" Applicants refer to paragraph [0036] of the specification. With regard to the recitation of "about 0.5 cm or less", Applicants refer to paragraph [0058], disclosing a tumor that was no larger than 0.5 cm in diameter.

In light of the above, Applicants request that the objections be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 29-35 and 40, 41, 43, and 44 stand rejected under 35 U.S.C. § 112, second paragraph as purportedly indefinite. Claim 29 is purportedly missing essential steps, with regard to the administration of CAPRI cells. Claim 29 is also rejected for the recitation of "administering the CAPRI cells into a cancer patient, wherein the cancer antigens are presented by activating APC obtained in step (a) to the naive T cells". However, Applicants wish to clarify that, with regard to the naivety of the PBMC used in step (b) of claim 29, the present invention does not test or select for naive cells. Rather, it is assumed that these cells are naive with respect to the cancer cells. By way of support, Applicants refer to results shown in Figure 1 (as showing no cell lysis without cascade priming). Such a test may also serve as a "functional test" for naivety with respect to the cancer. Accordingly,

Applicants submit that a step for selection for naive cells is not necessary. Instead, for example, a skilled artisan may use a function test or other test readily known in the art in order to determine naivete of cells, if even needed.

Regarding the recitation of "naive T cells" in claim 29, Applicants note that this phrase refers to the naive T cells comprised in the naive PBMC of step (b). By way of clarification, claim 29 is amended herein to recite "...wherein the cancer antigens are presented by the activated APC obtained in step (a) to PBMC comprising T cells of steb (b) in the context of a MHC complex ...".

Claim 41 is objected to for reciting "PMC" where base claim 29 recites "PBMC". Claim 41 is amended herein to make the phrases consistent.

Accordingly, in light of the above, Applicants request that the rejections under 35 U.S.C. § 112, second paragraph be withdrawn.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 29-35, 40, 41, 43 and 44 stand rejected under 35 U.S.C. § 112, first paragraph as purportedly failing to comply with the written description requirement. Applicants submit that the rejected claims are supported by the specification as filed. As noted, the present invention does not test or select for naive cells, but instead assumes that these cells are naive with respect to cancer cells. Further, the cancer antigens are presented by the activated APC obtained in step (a) to PBMC comprising T cells of steb (b) in the context of a MHC complex. Thus, the claims do not require a step directed to obtaining naive cells, and thus

support is not lacking for such a step. With regard to claim 40, as previously amended, Applicants amend this claim herein to remove reference to repeating a cycle of (d') and (d) up to ten times.

Applicants request that these rejections be withdrawn.

Rejections under 35 U.S.C. § 103

Claims 29-32, 34, 40, 41, 43, and 44 stand rejected under 35 U.S.C. § 103 as purportedly unpatentable over Babbitt in view of Gold, Wank, and Marzo.

Before turning to the cited references, Applicants provide the following regarding the present invention. Lymphocytes may be activated by various means. For example, lymphocytes may be activated with lectins and other soluble substances, such as interleukins, or with antibodies. This kind of activation leads to a proliferation of the T lymphocytes, but not cause a cytotoxic reaction against the body's own cells. Prior to destroying the body's own cells (*i.e.,* autologous cells), T lymphocytes must recognize that the cell is actually an autologous cell.

The T cell recognizes the presence of an autologous cell through contact with certain surface molecules referred to as HLA molecules (HLA-restricted detection). HLA molecules are found in all body cells. However, they are highly polymorphous and different for every human being, except for identical twins and so-called "HLA-identical" siblings. These HLA molecules bind cellular or viral or bacterial or mutated peptides of a cancer cell within the cell and present these peptides to the T lymphocytes. If the T cell recognizes only autologous

cellular peptides in the HLA molecule, the T cell will not be activated. However, if the HLA molecule presents a non-autologous microbial peptide or an autologous but mutated peptide, *e.g.* a cancer peptide, the T cell will kill the cancer cell or the infected cell.

The combined recognition of autologous HLA molecule and peptide is affected by the aß T cell receptor. The aß T cell receptor consists of an α and a ß chain and is highly polymorphous in every human being (there are trillions of different combinations in every human being). The aß T-cell receptor is part of the T cell receptor complex which further comprises the monomorphous CD3 chains y, δ, ε and ζ. The CD3 chains in each human being are basically identical.

Because the specific aß T cell receptor, which recognizes mutated peptides of a certain cancer species, is extremely difficult to detect, Babbitt discloses the choice of unspecifically activating the T cell with CD3 antibodies and the cytokines generated during activation (see column 3, lines 12-17: "the immunoreactive cells which have been generated independent of disease-specific antigens utilizing a mixture of nonspecific activators, i.e. autologous cytokines and a mouse monoclonal antibody, i.e. O M , as synergistic antibody"). The drawback of this activation is that the proliferation of T cells is induced, but does not induce the activation of the cytotoxic mechanism via the decisive a(3 T cell receptor.

This is actually demonstrated by Babbitt, as Figs. 2 through 6 only show the proliferation and cytokine production as a result of the activation. According to Fig. 7,

the cancer cell lines K562 and 769P, which express HLA only weakly or not at all, are killed. However, these cells can only be killed by NK cells (natural killer cells). Destruction of a tumor by OKT3-activated immune cells of the patient was not shown. With regard to the present invention, the Applicants could not detect any cytotoxic activity of CD3-activated PBMC against autologous cancer cells in the ^{51}chromium release assay. However, a profound cytotoxic effect of CAPRI cells was seen (see Fig. 1). Accordingly, the development of cytotoxic CAPRI cells would actually be blocked by an addition of the T3CS described by Babbitt.

The problem of CD3-activation is also not solved by an addition of cytokines, because CD3 chains are part of the T cell receptor complex. After activation of the CD3 chains, the internalization includes not only the CD3 chains but also the aß T cell receptors. Thus, a CD3-activation causes the paralyzation of the direct cytotoxic mechanism of cytotoxic CD3 T cells.

Only a minor percentage of NK cells carries the CD3 receptor. Thus, most NK cells are not affected by CD3-activation. NK cells are activated primarily by the "T3CS" cytokines produced by the method of Babbitt. However, NK cells are not suitable for the destruction of cancer cells of an autologous tumor, as they are inactivated by autologous HLA class I molecules which are expressed on basically all cancer cells.

Babbitt taken with Gold and Wank do not suggest the present invention. Babbitt and Gold use in both activation steps CD3 antibodies dissolved in cellular

medium, such that the CD3 antibodies bind to all T cells and the aß T cell receptor is internalized in all T cells. In the second activation step, the supernatant of the first activation, comprising the produced "T3CS" cytokines and not yet bound CD3 antibodies, is readjusted with additional CD3 antibodies to contain the original level of CD3 a ntibody. Thus, the second PBMC population is also treated with CD3 antibodies, and the cytokines obtained in the first activation step are merely boosting the proliferation. Gold describes that the underlying mechanism of a specific anti-tumor response generated via non-specific ex vivo activation with T3CS is not clear, as it was shown that T3CS expands only the number of memory T cells and obviously NK cells, "but not the naive T cell subset in patients". (see Gold, page 284, last paragraph). Further, Dey and North are cited noting that memory T cells can possibly be cytotoxic, "but on a delayed rather than immediate basis". As the CD3-activation suppresses the cytotoxic immune response, CD3 antibodies are used in immanent graft rejection in order to suppress the cytotoxic immune defense of the patient who received the graft.

In contrast to the methods of Babbitt and Gold, CAPRI cells kill autologous cancer cells directly within 18 hours, while CD3-activated PBMC show at most a minimal autologous cancer cell lysis (see Fig. 1).

According to the CAPRI method of the present invention, only the first PBMC population is activated with CD3. This is accomplished with CD3-antibodies fixated on the bottom of a plastic bottle. Antibodies dissolved in the

medium are removed by washing. The cytokines produced by activated T cells cause an activation of antigen presenting cells (APCs), predominantly monocytes and dendritic cells. (The CD3-activated T cells in this PBMC population have internalized their aß T cell receptors.)

It is important that the activated APCs present immunogenic cancer peptides in the HLA context to the non-activated T cells, which do not have internalized the aß T cell receptor. Naive T cells can be differentiated into cytotoxic and T helper effector cells only via the aß T cell receptor. By adding non-activated PBMC in the CAPRI method, T cells are added which are not activated via CD3 but via contact with APCs which present tumor-immunogenic peptides in the HLA molecule.

It is also important that the addition of the non-activated PBMC take place within a few hours so that APCs are in their activated state and an increased number of HLA molecules with the embedded mutated tumor peptides are expressed.

Due to the HLA molecules and the tumor immunogenic peptides presented in the HLA molecule of the APCs, the effector cells are produced via contact with the aß T cell receptor. These T effector cells kill the autologous cancer cells within 18 hours. Such effector T cells are not present in CD3-activated PBMC as demonstrated by the direct lysis test with cancer cells (cf. Fig. 1).

Cascade priming is the only method amongst the adoptive immune therapies wherein a differentiation of monocytes into dendritic cells occurs which present tumor-immunogenic peptides not only in HLA class I but also in HLA class II molecules. This is one of the key factors associated with the cytotoxic capacity of CAPRI cells.

The T effector cells activated via the aß T cell receptor can differentiate freshly added $CD14^+$ monocytes into $CD83^+$ dendritic cells, which best present the tumor-immunogenic peptides. The number of immature $CD14^+$ monocytes, with their lesser ability for presentation, is reduced.

In contrast, CD3-activated PBMC are not able to differentiate $CD14^+$ monocytes into $CD83^+$ dentritic cells. Accordingly, the number of $CD14^+$ monocytes remains unchanged (see Fig. 2). In the CAPRI method, the increase of dendritic cells results also in the activation of $CD4^+$ helper T cells and not only of cytotoxic $CD8^+$ T cells via the op T cell receptor. Those $CD4^+$ helper T cells are necessary for a strong cytotoxic HLA-restricted immune response.

A further problem found in the art was the fact that epiphelial cancer cells tend to express only rudimentarily HLA molecules of class I and only rarely HLA class II molecules. This problem is not addressed or solved by Babbitt or Gold. It is known that a rudimentary expression of HLA molecules leads to the inactivation of T lymphocytes which have infiltrated the tumor (so-called TIL or tumor infiltrating

lymphocytes). This inactivation damages TIL permanently so that they cannot expand any further.

However, CAPRI cells are largely resistant against an inactivation by tumor cells. After destroying one tumor cell layer, they can be re-administered to a new tumor cell layer, where they destroy this new layer without the addition of cvtokines and other substances. They are able to do this due to a further ability of CAPRI cells. Upon contact of cancer mils with CAPRI cells, the expression of HLA class I and HLA class I I molecules is stimulated and/or enhanced in the cancer cells, and thereby also the expression of tumor-immunogenic peptides. In contrast, CD3-activated cells do not boost the HLA expression of cancer cells (see Fig. 3).

Thus, in the CAPRI method, not only cytotoxic $CD8^+$ T cells but also $CD4^+$ T cells are activated by epithelial cancer cells which usually express HLA class II only weakly or not at all.

Marzo does not remedy the deficiencies of the other cited references. Marzo shows that despite an ubiquitary presence of tumor antigen in the lymphatic system, immune cells are not capable of developing a sufficient cytotoxic response. Marzo concluded that it would be futile to remove enlarged lymph knots near the tumor, because tumor antigen is present in the whole lymphatic system. For the production of CAPRI cells, the cells are obtained from blood and not from the lymphatic system. Tumor markers are partially tumor antigens and are known for decades. Marzo suggests increasing the tumor load for CD8 cytotoxic

cells in order to obtain a stronger proliferation of cells that had already been in contact with tumor antigen. However, in order to follow the recommendations of Marzo, lymph vessels leading away from the tumor would have to be identified, the T lymphocytes isolated from those and re-stimulated with tumor. In principle, this has already been tried with tumor-infiltrated lymphocytes without success, as these cells were already inactivated.

Johnson discloses a method of binding antibodies to a radionuclide in case antibodies are produced which bind tumor-associated antigens. However, such antibodies would not bind to tumor peptides that are presented by HLA molecules, but to "tumor-associated antigens". Tumor-immunogenic peptides that are presented in the HLA complex are not associated but tumor specific. According to this method, HLA-restricted antibodies have to be developed each time for every patient separately due to the high polymorphism of the HLA system.

In summary, all attempts to develop an improved treatment for cancer patients by trying to generate HLA restricted cytotoxic lymphocytes from the tumor or from the lymph vessels leading away from the tumor (Matzo) failed. Further, the method of Babbitt would inactivate CAPRI cells , as the addition of T3CS supernatant would lead to the internalization of op T cell receptor and therefore to blocking the non-HLA-restricted cytotoxic activity due to the presence of OKT3 antibodies.

Accordingly, taking the cited references in combination, Applicants submit that one of skill in the art would not arrive at the presently claimed invention. The cited references fail to teach or suggest the present adoptive immune cell therapy.

Claims 29 and 33 stand rejected under 35 U.S.C. § 103 as purportedly unpatentable over Babbitt in view of Gold, Wank, and Marzo, and in further view of Granger and Johnson.

Babbitt, Gold, Johnson and Marzo are discussed above. The method by Granger tries to utilize the strong non-HLA-restricted immune response against allo-antigens for the immune response against cancer cells. As the immune cells of a different donor attack all body cells of the patient, particularly after "pre-sensibilisation" in the MLC (graft-versus-host effect), they are administered close to the tumor intralesionally as a "cytoimplant" in order to achieve the main effect there and to not harm other tissues besides the tumor-affected pancreas (see the paper by Chang *et al.*, e nclosed herewith). However, in contrast to the presently claimed CAPRI approach, the MLC reaction observed is not an HLA-restricted immune response. Instead, it is primarily directed against any tissue antigens from the non-HLA-compatible host and not against microbial peptides or cancer peptides.

The mean survival time for glioblastoma is about one year. An allo-immune cell therapy according to Granger is not able to prolong significantly the survival time. Further, the later publication by Chang *et al.* (enclosed herewith) on Mixed Lymphocyte Culture "Cytoimplantates" for pancreatic carcinoma resulted only in a

mean survival time of 13.2 months in 8 patients. Here too, no prolonged survival time was observed.

Further, the method according to Granger is not directed against tumor-specific HLA-restricted peptides and is not capable of differentiating naive T lymphocytes into cytotoxic and helper T effector cells. Chang *et al.* show for 8 patients with pancreatic carcinoma that no significant extension of survival time could be reached with this method. Therefore, a combination of these methods by adding T3CS according to Babbitt *et al.* into MLC according to Granger, would not have an expectation of success. Moreover, it is known from numerous bone marrow grafts that allogenic immune cells administered in a certain amount lead to the death of the receiving patient.

Applicants submit that present CAPRI method with the addition of non-stimulated PBMC after a few hours is not obvious in view of the cited references. Surprisingly, the CAPRI method according to the present application leads to a fast maturation of monocytes into dendritic cells (see Fig. 2).

In light of the above, Applicants request that the rejections under 35 U.S.C. § 103 be withdrawn.

CONCLUSION

If there are any questions regarding this amendment or the application in general, a telephone call to the undersigned would be appreciated since this should expedite the prosecution of the application for all concerned.

If necessary to effect a timely response, this paper should be considered as a petition for an Extension of Time sufficient to effect a timely response, and please charge any deficiency in fees or credit any overpayments to Deposit Account No. 05-1323 (Docket #99380.B090019).

Respectfully submitted,

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